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Conference Report: The 6th International Symposium on Waterborne Pathogens

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EXPERTS FROM AROUND THE WORLD CONVENED TO DISCUSS THE THREAT OF VARIOUS PATHOGENS AND TO PRESENT RECENT RESEARCH THAT EXEMPLIFIES THE INDUSTRY'S CONTINUED VIGILANCE AGAINST DISEASE OUTBREAKS.

AWWA's 6th International Symposium on Waterborne Pathogens (ISWP) was convened in Savannah, Ga., in April 2015. The meeting brought together 141 attendees from six countries to discuss pathogen occurrence, detection, and treatment in water. Waterborne diseases are still a potential significant threat to public health, even in developed countries with well-regulated water industries, so continued vigilance is necessary. Much of the symposium's focus was on *Legionella*, *Cryptosporidium*, and *Naegleria fowleri* (N. *fowleri)*, but *Pseudomonas aeruginosa (P. aeruginosa)*, norovirus, and toxigenic *Escherichia coli (E. coli)* were also discussed in addition to fecal indicator bacteria, pathogen risk modeling, water reuse, pathogens in wastewater, and antibiotic-resistant bacteria. This article describes

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some of the information that was presented during the symposium and summarizes much of the discussion that ensued after the speakers finished presenting, and during two town-hall-style sessions. For further information about the 6th ISWP presentations and future symposiums, access the ISWP proceedings at www.awwa.org/store/productdetail.aspx?productid=50657503 ($75 for AWWA members, $125 for nonmembers) or contact the corresponding author.

INTRODUCTION

There have been substantial improvements in preventing waterborne disease and outbreaks in developed countries over the past two decades. Consequently, it can be easy to get complacent about pathogens in drinking water in the absence of large, well-publicized waterborne disease outbreaks such as Milwaukee, Wis., in 1993 and Clark County, Nev., in 1994 (Goldstein et al. 1996, MacKenzie et al. 1994). However, waterborne microbial diseases are still a cause of substantial morbidity (and some mortality), even in developed countries; for example, an estimated 4 million to 16 million cases of waterborne gastroenteritis occur annually in the United States (Colford et al. 2006, Messner et al. 2006). Therefore, persistent vigilance is required to continue controlling the risks posed by waterborne pathogens.

Pathogens in drinking water have received considerable media attention recently in the United States. Over the past 10 years, *Legionella* has become the most frequent cause of waterborne disease outbreaks in the United States, as shown in Figure 1 (Beer et al. 2015). Also, a cryptosporidiosis outbreak in Oregon in 2013 (Oregon Health Authority 2014) was the first time since Clark County, Nev., over 20 years ago in which a US disease outbreak was linked to municipal drinking water. And setting an unwelcome precedent, the first cases of fatal primary amoebic meningoencephalitis (caused by *N. fowleri)* linked to treated municipal drinking water were reported in 2012 in Louisiana (Yoder et al. 2012). Therefore, it is not surprising that all three of these pathogens were discussed in detail at the 6th ISWP. This symposium, which is organized every three to five years by AWWA's Organisms in Water Committee, provides a forum to discuss all aspects of pathogen occurrence, detection, and treatment in water.

The 2015 meeting hosted 141 attendees from six countries—the United States, Japan, the United Kingdom, Canada, the Netherlands, and New Zealand; attendees represented water utilities, academia, public health and other government agencies, equipment manufacturers, and environmental consulting firms. Speaker affiliations and locations are shown in Table 1. In addition to sessions and discussions about the three pathogens noted here, two days of intense, single-track sessions covered *P. aeruginosa*, Norovirus, toxigenic *E. coli*, fecal indicator bacteria, pathogen risk modeling, water reuse, pathogens in wastewater, and antibiotic-resistant bacteria.

> "Excellent topics and a very valuable symposium. No other AWWA event features as much information on water microbiology. Please continue this symposium."—attendee at the 6th ISWP.

PUBLIC HEALTH OVERVIEW

Michael Beach from the Centers for Disease Control and Prevention (CDC) gave the opening keynote address, discussing the CDC0's primary drinking water concerns. The CDC's focus areas are emerging pathogens, disinfection-tolerant pathogens, new transmission routes beyond drinking water, aging water treatment and delivery infrastructure, pathogens in premise plumbing, and unregulated or under-regulated small drinking water systems. Unlike the US Environmental Protection Agency (USEPA), the CDC has an advisory function rather than a regulatory role. The CDC's responsibility is to identify gaps in health information; help fill those gaps; and improve national, state, and local capacity for waterborne disease surveillance and reporting. As Beach discussed, new causes or sources of outbreaks that the CDC will add to the national surveillance system beginning in 2016–2017 are cyanotoxins and health care–associated infections (i.e., nosocomial infections) that are linked to hospital plumbing systems.

The water within a building's plumbing system (i.e., premise plumbing) can be very different from the Water that is originally delivered from a treatment plant because of water aging, temperature, loss of disinfectant residual, and piping configuration, all of which may lead to growth of biofilms within the pipes. These biofilms can then provide the ideal habitat for survival and proliferation of certain pathogens (Figure 2). Recent data suggest that premise plumbing pathogens such as *Pseudomonas* spp., nontuberculous mycobacteria (NTM), and *Legionella* kill more people in the United States than waterborne fecal-oral-type pathogens. Although toxin-producing cyanobacteria were not discussed at this symposium because they are not "pathogens," a well-publicized do-not-drink order in 2014, legislative action in Washington, D.C., and the release of federal cyanotoxin health advisories (USEPA 2015) highlight the increasing importance of this group of microorganism-generated contaminants.

DISTRIBUTION SYSTEM AND PREMISE PLUMBING PATHOGENS

Legionella pneumophila has become the dominant cause of documented waterborne disease outbreaks in the United States over the past 10 years (Beer et al. 2015), in part because of improved diagnosis and reporting as well as increased populations of susceptible individuals. *Legionella* is already regulated at municipal treatment plants under the Surface Water Treatment Rule (treatment technique), but outbreaks are primarily linked to building water systems that are beyond the control of water utilities. The microorganism is a particular concern in hospitals and nursing homes where susceptible individuals are likely to be concentrated. More abstracts on *Legionella* were submitted for the symposium than for any other pathogen, reflecting the water community's renewed focus on this pathogen.

USEPA was represented at the meeting and has been working with a multi-agency taskforce (USEPA Office of Water and Office of Research and Development, CDC, Association of State Drinking Water Administrators) to produce a document on treatment technologies for controlling *Legionella* in building water systems (draft document expected publication in late 2015). These treatment technologies include on-premise application of common drinking water disinfectants (e.g., chlorine, chloramines, chlorine dioxide), copper-silver ionization, and ultraviolet (UV) irradiation. However, maintaining a clean distribution system within buildings is critical because accumulated biofilms reduce the efficacy of these disinfectants. Clogging and filter failures seem to outweigh the benefits of using point-of-use filtration for *Legionella* control in building plumbing systems. Ultimately, flushing systems with high-temperature water (>66°C [150°F]) and shock chlorination may be necessary for emergency disinfection of systems with established problems. Maintaining a higher water temperature (e.g., ≥49°C [120°F] in residential water heaters) can minimize growth of *Legionella* in a domestic hot water system, but the balance between *Legionella* control versus the increased risk of scalding and higher energy costs needs to be considered.

Comments from audience members during the session on *Legionella* centered on the difficulty in applying municipal water treatment disinfection practices to building plumbing systems; dedicated disinfection requirements specific for building plumbing systems are needed. It was also suggested that the US water community learn from European experiences and expertise, since some countries such as the Netherlands and United Kingdom have already done a lot of work on *Legionella* control in building plumbing systems.

Although primary amoebic meningoencephalitis (PAM) caused by *N. fowleri* is rare, any disease that prompts headlines such as “Brain-Eating Amoeba in Your Drinking Water” is understandably going to be a concern. Three presentations covered N. *fowleri* in detail, describing recent drinking water-associated PAM cases, recent N. *fowleri* and thermophilic amoeba contamination of drinking water systems in Louisiana, and new sampling and detection methods (see the group of photographs on page 30). As discussed in the opening address, the CDC’s role for PAM is to provide 24/7 clinical guidance and diagnostic consultation to health professionals. The overall incidence of PAM cases so far does not appear to have increased with climate change, but the geographic distribution of cases is spreading. The last few years have seen the first PAM infections in northern states, the first infections linked to nasal irrigation with tap water, and the first survivor of PAM since 1978. In 2013, the CDC got permission from the US Food and Drug Administration to use an investigational medication, miltefosine, to treat a 12-year-old child, who survived.

Recent PAM deaths traced to the use of neti pots and a water slide, and detection of N. *fowleri* in source and treated waters in Louisiana, have prompted the development of a *Naegleria* response plan in that state that is based on experiences from Australia. The plan requires maintaining a chlorine (or chloramine) residual of not less than 0.5 mg/L at all points in the distribution system, monitoring disinfectant residual at 25% more sites than are required for total coliform monitoring, and submitting a nitrification control plan for those systems using chloramination. Monitoring conducted using a combination of grab samples and ultrafiltration, thermophilic amoeba culture, microscopic examination, and a polymerase chain reaction (PCR) assay demonstrated that most detections of thermophilic amoeba in the Louisiana drinking water surveillance project were associated with disinfectant residuals of less than 0.5 mg/L. Audience discussion centered on qualifying the recent Louisiana cases as not really being linked to treated water because there was no detectable disinfectant residual and there were dead spots in the distribution system serving the victims’ locations. This discussion raised a question: should treated water that no longer contains residual disinfectant and that may have been aging in the distribution system for a considerable period still be considered "treated"? Properly treated water that contains residual disinfectant and is not stagnating in the distribution system should not present a *Naegleria* risk.

> Recent data suggest that premise plumbing pathogens kill more people in the United States than waterborne fecal-oral-type pathogens.

Although *Pseudomonas* is not typically considered as a primary waterborne pathogen, some species of *Pseudomonas* can cause opportunistic infections, and a few presentations reported on the latest findings on this potentially important but somewhat overlooked organism.

P. aeruginosa is one of most common causes of nosocomial infections. As reported at the symposium, in one study at four health-care facilities, 1 to 18% of tap water samples were positive for *P. aeruginosa*, 11 to 50% of patient strains were the same as tap water strains, and 30 to 50% of infections in intensive care units were associated with tap water. As water conservation measures become more widespread, devices such as electronic faucets (e-faucets) are being promoted. However, 14 to 31% of e-faucets in the four health-care facilities were positive for *P. aeruginosa*, depending on the location of the hot/cold mixer. Therefore, there is a balance between saving water and potentially creating conditions for pathogen growth in water lines.

An interesting study on opportunistic pathogens in distribution systems in the Netherlands was also presented. Unlike in the United States and many other countries, finished drinking water in the Netherlands is not chlorinated. The current order or priority for investigating opportunistic pathogens in the Netherlands is *Legionella*, followed by NTM, *P. aeruginosa, Aspergillus fumigatus, Stenotrophomonas*, and *Acantbamoeba.* Interestingly, NTM are not typically detected in nonchlorinated Netherlands waters with low assimilable organic carbon, but they are detected in US chlorinated waters. It was suggested that chlorine may inactivate competing bacteria in drinking water and potentially promote the growth of NTM.

CRYPTOSPORIDIUM

More than 20 years after the Milwaukee outbreak, the drinking water community is still trying to determine the true risk to public health from *Cryptosporidium* in drinking water. As with all previous pathogens symposia in this series, *Cryptosporidium* was well represented. The first symposium was held four years after the Milwaukee cryptosporidiosis outbreak of 1993. The 2015 symposium also had significant interest because of an outbreak in 2013 that was traced to a small surface water utility in Baker City, Ore. With 119 confirmed cases and an attack rate of 28% among a population of about 10,000 people, it was the first US cryptosporidiosis outbreak linked to municipally treated water in more than 20 years (Figure 3). Following a three-week boil-water notice and the resumption of normal service, the utility installed UV treatment and implemented more stringent watershed protection measures.

The CDC's Beach also discussed *Cryptosporidium* during his opening address, describing a 400% increase in cryptosporidiosis cases over the past 15 years. This increase is thought to be a result of a combination of an actual increase in disease incidence as well as increased reporting and better diagnosis. He emphasized the value and necessity of molecular characterization, particularly of sporadic (non-outbreak) strains, and suggested following the molecular epidemiology and tracking model used in the United Kingdom. Therefore, a national sequence-based molecular surveillance system for *Cryptosporidium* (CryptoNet) is in development by the CDC.

The ISWP was held just two weeks after the start of the second round of large system *Cryptosporidium* monitoring under the LT2ESWTR. Although USEPA Method 1622/1623/1623.1 is the mandated method, there is still a need for alternative oocyst detection methods. A novel method presented during the symposium involved detecting oocysts in biofilm scrapings from river rocks or on adapted microscope slides placed in surface water. Benthic-rock biofilm sampling can be useful for long time-scale monitoring (months) to identify point sources of oocysts along the length of a waterway. For shorter time scales (days), oocyst detection on slide biofilms was comparable to Method 1623 filtration-based monitoring, and the slide method was found to be less expensive.

New developments in a cell culture-based infectivity assay for *Cryptosporidium* were also presented. The proportion of cell culture infectious oocysts in a sample increased from ~10% using the established method to ~60 to 70% with the refined method. This assay was evaluated using spiked finished drinking water samples, and infections were detected in 70% of cell culture wells inoculated with concentrates from matrix spikes containing just a single oocyst, and 90% of wells for matrix spikes containing three oocysts. This assay may provide more refined *Cryptosporidium* risk assessments and is an additional tool in protecting public health. The presentation ended with a discussion on the significance for previous monitoring studies and risk assessments based on earlier, less-sensitive infectivity methods. The prospect of repeating large-scale surveys of finished water was discussed without resolution.

METHODS

Numerous microbial analysis tools were discussed and described during the meeting:

- Using ultrafiltration and realtime PCR to detect *N. fowleri*
- Using 16S and 18S rDNA metagenomics for detecting *N. fowleri* and associated bacteria in drinking water
- Drinking water microbiome characterization by 16S rDNA amplicon sequencing
- RNA-based detection (rather than DNA-based) for increased detection sensitivity and viability assessment of fecal indicators
- Detecting fecal indicator bacteria by qPCR
- New microbial source-tracking markers, including promising enteric virus source-tracking tools

But not all of the promising new methods were molecular. As noted previously, a simple, low-cost method was reported that used benthic rock and microscope slide biofilms to adsorb *Cryptosporidium* oocysts in surface water. Additionally, a new optimized cell culture method was reported to significantly increase the detection of infectious *Cryptosporidium* oocysts.

RISK ASSESSMENT

Quantitative microbial risk assessment (QMRA) has become increasingly popular to characterize and quantify the risk to public health from pathogens in water. Although QMRA-based risk assessment tends to be site-specific, requiring a model for each site or situation, a model for developing public health triggers for drinking water was presented. But risk-assessment models are only as good as the data that are fed into them. A presentation on norovirus dose-response information concluded that because of incorrect assumptions about virus particle aggregation and host susceptibility, most risk calculations overestimate the risk of infection at low doses of norovirus, possibly by as much as three orders of magnitude. More work is needed to reconcile such disparate endpoints. But since one requirement to improve models is better data from human-feeding trials with disaggregated viruses, such work may be difficult.

WASTEWATER AND REUSE

A growing interest in alternative water supplies is being driven by prolonged drought in some regions, increasing populations, and competing demands for water. Consequently, potable reuse of wastewater (both direct and indirect) is now a serious consideration for many municipalities. Pathogen control is the most critical aspect of direct potable reuse because pathogens will always be present in wastewater, and they could have an immediate impact on public health if treatment barriers are not maintained. Characterizing wastewater is important for determining the efficacy of treatment. One Canadian study reported detecting adenoviruses in wastewater at higher concentrations than other enteric viruses. Physical microfiltration appeared to be more efficient for virus removal than traditional secondary treatment and inactivation by UV irradiation. A study of decentralized water reuse systems found that they could produce good-quality effluent for nonpotable reuse, but small-scale, low-tech systems (e.g., aerobic septic systems) achieved lower enteric microbe reductions and more variable reductions versus higher-tech systems (e.g., membrane bioreactors with advanced oxidation). The WateReuse Research Foundation sponsored a project to determine a set of potable reuse criteria that are protective of public health and to evaluate the ability of potable reuse trains to meet these criteria. As the water reuse effort moves forward, public health criteria may be met by defining treatment redundancy and robustness of each portion of the treatment train.

TOWN HALL DISCUSSIONS

To give attendees a break from the standard meeting format of one technical presentation after another but still keep the discussion and flow of information moving, two town hall-type sessions were organized, each around a separate theme. The sessions provided attendees with the opportunity to discuss topics in a less formal setting, including around lunch tables. Judging from comments received after the symposium, many attendees found these sessions to be useful and informative.

Town hall discussions were held on both days and focused on two broad topics: (1) pathogen monitoring in drinking water and (2) opportunistic pathogens in premise plumbing. The importance of the second round of LT2 monitoring was discussed, especially the added value of such monitoring programs. The implementation of the updated Method 1623.1 and the potential impact of using different LT2ESWTR-approved methods on bin classifications were debated. There was also a discussion about the potential economic impacts of research on consumers. If research results cause water rates to increase, it is essential that they be accurate and up to date. Thus, it is necessary to confirm and validate research results prior to using them to support regulation or policy. Another topic that spurred a lively discussion was virus-monitoring programs for drinking water and the influence of virus stock preparation (e.g., minimizing viral particle aggregation) on the accuracy of enteric virus QMRA models.

The majority of the discussion about opportunistic pathogens in premise plumbing focused on *Legionella*, NTM, and *P. aeruginosa.* Conversations were spurred by a variety of questions:

- How is the concept of contamination different for opportunistic pathogens in premise plumbing compared with enteric microbes?
- Can risk be established using presence/absence data only?
- Is quantification (microbe concentration) necessary?
- What type of samples should be analyzed—bulk water or biofilms?
- Can a building's plumbing system be effectively disinfected?
- Who is responsible for this disinfection?
- Who is responsible for preventing premise plumbing contamination?

The ensuing discussions attempted to answer these and other questions regarding opportunistic pathogens in premise plumbing. The point was made that these pathogens cannot be considered "contaminants" in the same way as enteric pathogens since they are not introduced from a contaminating source. They are always present, but whether they proliferate and create disease depends on distribution system and premise system management practices. Routine monitoring for opportunistic pathogens in premise plumbing will not be easy because each building is likely to have unique engineering characteristics and building tenants with varying susceptibilities to infection, which would limit the applicability of a one-size-fits-all monitoring program. Some of those participating in the discussions said they wanted to see quantitative microbial data so that the effectiveness of control measures could be tracked. However, other participants saw limited value in quantification because host susceptibility plays a much bigger role in severity of infection and risk assessment than organism concentration.

In the Netherlands, all hospitals and communal buildings are required to have extensive control plans for *Legionella.* However, these plans have not made any difference in disease incidence, leading some to conclude that drinking water systems may not be the primary cause of infection. Other sources, such as cooling towers, may be more important. Public health authorities in Germany have implemented control requirements for cooling towers, and they are now waiting to see whether disease incidence is reduced.

There was also discussion about the importance of considering the ecology of all microbes in premise plumbing systems, since they will mostly be components of a biofilm, and biofilms involve complex interactions between microbes. Focusing on individual microbes in isolation, without regard for their interaction with other community members, is likely to result in gaps in our understanding of the problem. The discussion concluded with a general consensus that the water community is moving into an area of shared responsibility with utilities and building owner/operators working together on premise plumbing issues.

> Public communication about pathogens in water is likely to become a lot more important by the time the next ISWP is held.

CONCLUSIONS

In the absence of large municipal waterborne disease outbreaks, there may be a tendency for policymakers, politicians, and the general public to assume that there is no waterborne disease in the United States. Consequently, there are limited resources for waterborne pathogen studies and research. To the contrary, people in the United States and other developed countries are still at risk from pathogens in drinking water with up to 16 million annual cases of waterborne gastroenteritis in the United States, according to some estimates. However, the face and pattern of waterborne microbial diseases have changed. Drinking water, swimming pools, waterparks, recreational lakes and rivers, large community buildings (hospitals, nursing homes, schools, apartment complexes), cooling towers, fountains, produce misters at the grocery store, and cultural ablution practices all present potential opportunities for pathogen transmission through water.

This increasingly complex picture of waterborne disease and public health was highlighted by the lengthy discussion during the symposium about the need to promote improved public health through communication and outreach. This outreach must also extend to policymakers. Although considerable time and effort have already been devoted to public outreach, the messages can be complex and confusing. For example, the message from water utilities is that the water supply is safe and reliable, which is true most of the time. However, those same utilities and public health agencies may also tell the public not to use tap water for nasal irrigation, with medical devices, or to rinse or store contact lenses. Therefore, public communication about pathogens in water is likely to become a lot more important by the time the next ISWP is held.

The 6th ISWP was highly successful and added significantly to the knowledge base of this important topic. According to ISWP attendee feedback, some changes that may need to be considered for the next symposium include a stronger emphasis on utility perspectives and experiences and discussions of a wider diversity of pathogens (notably enteric viruses and other Contaminant Candidate List pathogens). The timing and agenda of the 7th ISWP will be a topic for discussion at the next AWWA Organisms in Water Committee meeting at the 2015 Water Quality Technology Conference & Exposition (WQTC), which is scheduled for

Nov. 15–19, 2015, in Salt Lake City, Utah. More information about WQTC can be found online at www.awwa.org/wqtcl5.

Biography

Rochelle has been a microbiologist at MWDSC for 20 years, initially at the laboratory bench and then as a manager in the Water Quality Laboratory for the past 13 years. He leads the Source Water Microbiology Team and previously led the Methods Development Team as principal microbiologist. His experience at MWDSC includes pathogen monitoring, regulatory compliance, improved methods to detect and identify pathogens, developing Cryptosporidium infectivity assays, assessing treatment plant performance, evaluating effectiveness of disinfection, monitoring and investigating invasive mussels, and cyanotoxins. He has organized or helped to organize workshops and symposia on a variety of water quality issues including Contaminant Candidate List pathogens and invasive species and has served on numerous committees and workgroups focused on the microbiology of drinking water.

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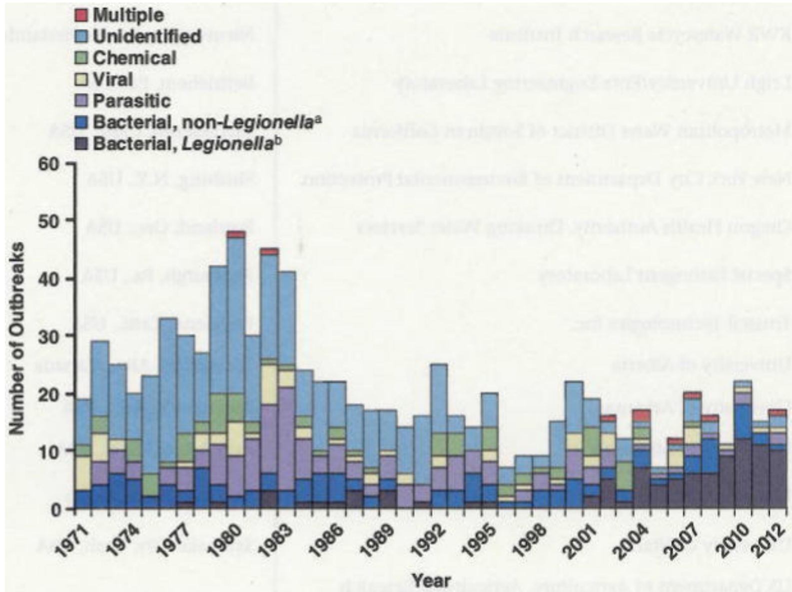


FIGURE 1.

Number of waterborne disease outbreaks associated with drinking water (n = 885), by year and etiology—United States, 1971–2012

Source: Beer et al. 2015

[a]Includes all bacteria, other than *Legionella*

[b]Legionellosis outbreaks were first reported to WBDOSS in 2001. Legionellosis outbreaks prior to 2001 were added retrospectively during the 2007–2008 reporting period.

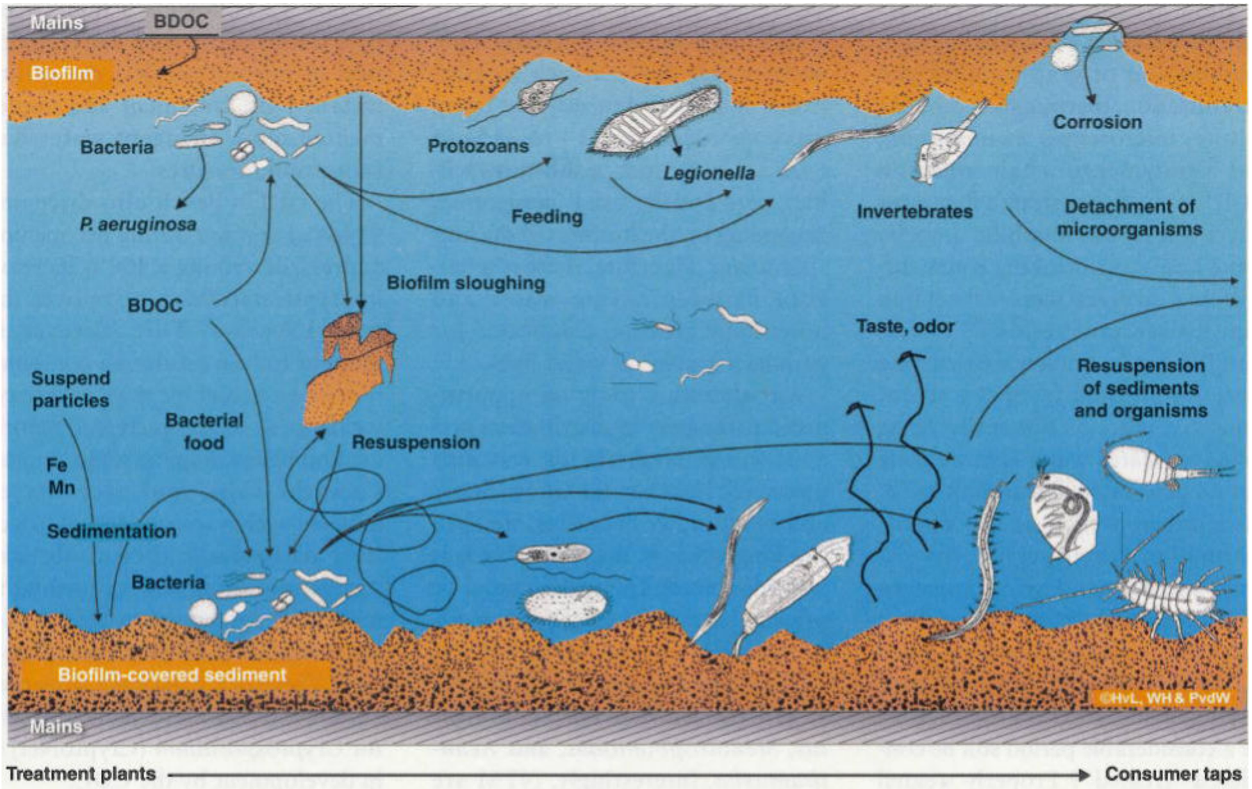


FIGURE 2.

Organisms and processes associated with biofilm and potential regrowth of organisms in pipes

Source: © 2015 van Lieverloo, Hoogeboezem and van der Wielen. Used with permission. BDOC—biodegradable dissolved organic carbon, Fe—iron, Mn—manganese, *P. aeruginosa*—*Pseudomonas aeruginosa*

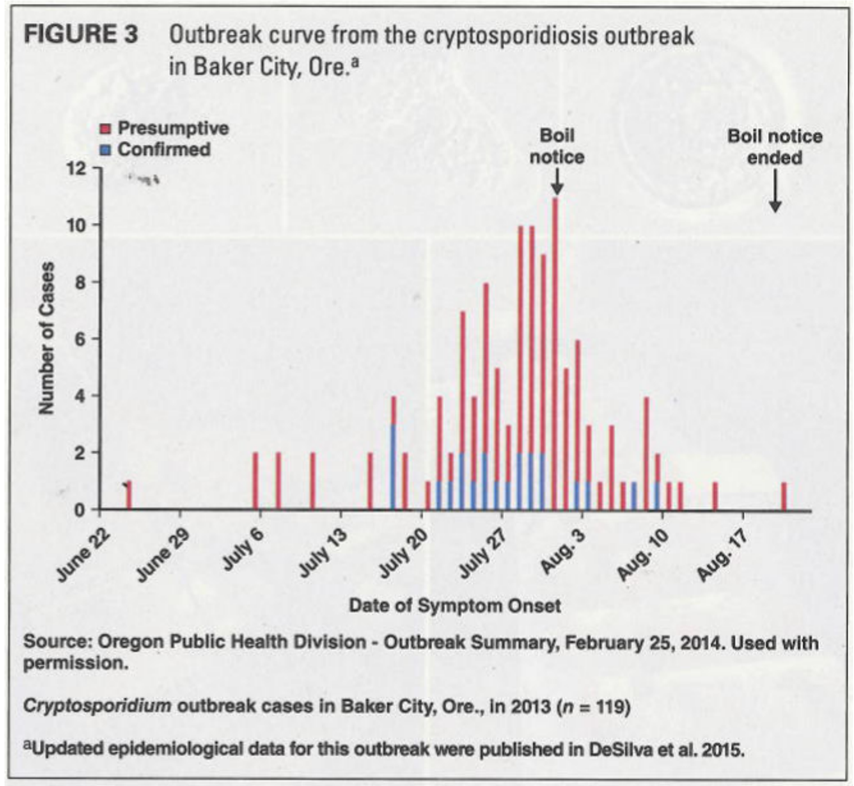


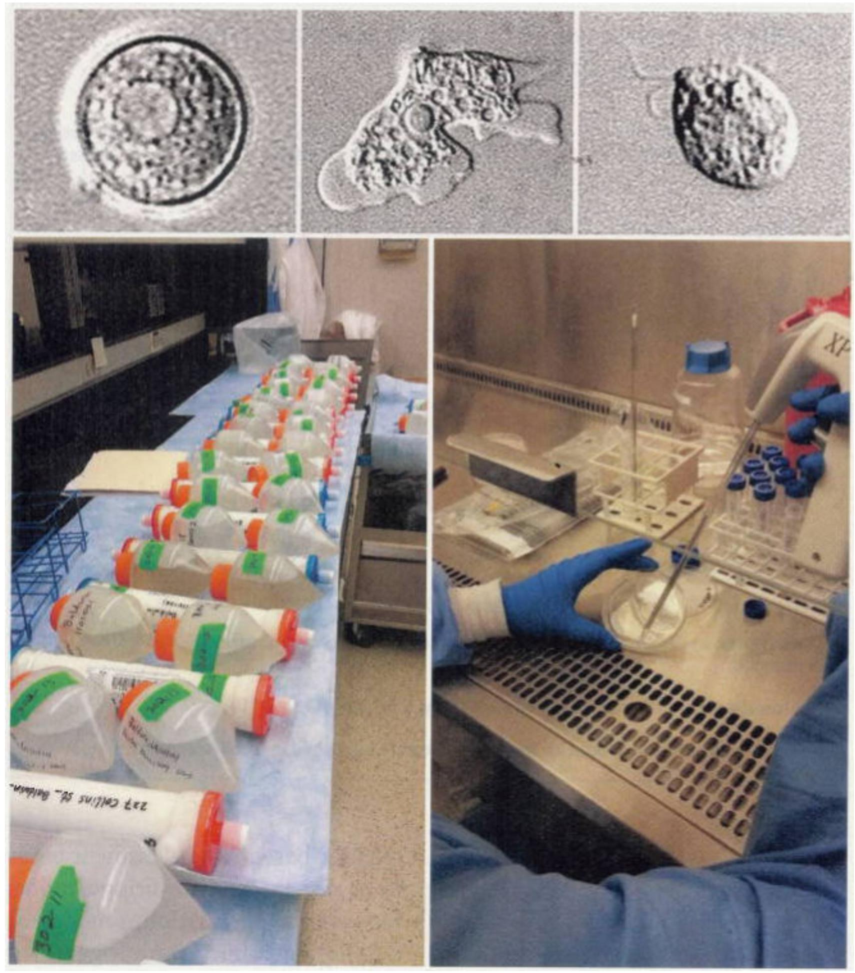
FIGURE 3.

Outbreak curve from the cryptosporidiosis outbreak in Baker City, Ore.[a]

Source: Oregon Public Hearth Division – Outbreak Summary, February 25, 2014. Used with permission.

Cryptosporidium outbreak cases in Baker City, Ore., in 2013 (n = 119)

[a]Updated epidemiological data for this outbreak were published in DeSilva et al. 2015.



Components of analytical method for *Naegleria fowleri*
Photos courtesy of Jennifer Clancy. Used with permission.

TABLE 1

Affiliations of speakers at the 6th International Symposium on Waterborne Pathogens

Organization	Location
Centers for Disease Control and Prevention	Atlanta, Ga., USA
City of Ottawa	Ottawa, Ont., Canada
Corona Environmental Consulting	Scituate, Mass., USA
École Polytechnique of Montréal	Montréal, Quebec, Canada
EPCOR Water Services Inc.	Edmonton, Alta., Canada
Health Canada	Ottawa, Ont., Canada
KWR Watercycle Research Institute	Nieuwegein, the Netherlands
Leigh University/Fritz Engineering Laboratory	Bethlehem, Pa., USA
Metropolitan Water District of Southern California	Sacramento, Calif., USA
New York City Department of Environmental Protection	Flushing, N.Y., USA
Oregon Health Authority, Drinking Water Services	Portland, Ore., USA
Special Pathogens Laboratory	Pittsburgh, Pa., USA
Trussell Technologies Inc.	Pasadena, Calif., USA
University of Alberta	Edmonton, Alta., Canada
University of Arkansas	Fayettevitle, Ark., USA
University of Michigan	Ann Arbor, Mich., USA
University of Tokyo	Bunkyo, Tokyo, Japan
University of Utah	Salt Lake City, Utah, USA
US Department of Agriculture, Agricultural Research Service Environmental Microbial and Food Safety Laboratory	Washington, D.C., USA
US Environmental Protection Agency	Washington, D.C., USA
Virginia Polytechnic Institute and State University	Blacksburg, Va., USA